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BIOLOGICAL BULLETIN

THE INFLUENCE OF THE AMOUNT OF INJURY UPON THE RATE AND AMOUNT OF REGENERATION IN MANCASELLUS MACROURUS (GARMAN).[1]

MAX M. ELLIS.

I. Introduction.

Until Dr. Zeleny announced the results of his experiments on the fiddler crab, *Gelasimus*, and the brittle star, *Ophioglypha*, it was generally thought that an increase in the amount of injury would decrease the ability to repair injury, that is, it would retard regeneration. In these two forms he found that an increase in the degree of injury produced a corresponding increase in the rate of regeneration. Later he established the same principle as true for the common crawfish,[2] *Cambarus propinquus*.

From the crawfish experiment he concludes that "in series with the greater degree of injury each chela regenerates more rapidly than the single removed chela of the series with the lesser degree of injury." These three experiments present the idea that an increase in the amount of injury accelerates rather than retards regeneration. However, it is probable, as is indicated by Dr. Zeleny's work on the brittle star, that there is a degree of injury, a limit, beyond which this is not the case. In the work just mentioned,[3] when all five of the arms were removed the regeneration was slower than when four were removed.

The present experiments were made with the object of contributing some quantitative data concerning the relation of the amount of injury to the rate and amount of regeneration. The

[1] Contribution from the Zoological Laboratory of Indiana University. No. 85.

[2] *Jour. Ex. Zoöl.*, Vol. II., No. 3, '05.

[3] *Jour. Ex. Zoöl.*, Vol. II., No. 1.

form *Mancasellus* was chosen because it is abundant in this region. It regenerates lost parts readily and as each leg has a breaking joint at the coxal-thoracic articulation it is possible to make all operations uniform. The results obtained are, in a measure, parallel with Dr. Zeleny's work.

II. Methods.

The isopods used in these experiments were taken in a stream near the Indiana University campus at Bloomington, from a part not over three hundred feet in length. In this distance it is probable that they had been subjected to the same general conditions previous to the experiments.

Set I. — For Set I. several hundred specimens were collected on October 3, 1906, from which twenty normal males measuring between 10–13 mm. were selected. These were divided into four series. In series A a "standard" injury was established by the removal of the right sixth walking leg. In each of the remaining three series the operation was the infliction of the "standard" injury plus an "added" injury. As "added" injury the right fifth walking leg was removed in series B, the right fifth, fourth and third in series C and all of the right thoracic appendages including the cheliped in series D. The operation was made in each series by pinching the tip of the appendage to be removed till the animal cast it off at the breaking joint. The above system was repeated daily on a fresh catch of Isopods for five days, that is, until October 8, 1906, when the completed set consisted of four series of twenty-five individuals each. Each specimen was kept in a twelve-ounce saltmouth bottle, which was inclined a few degrees from the horizontal by the mouth resting on an inch block. The water was changed every six days. As food, the partially decayed leaves of the common elm were supplied. An excess of leaves was always present. Fourteen days after the date of operation each individual was killed.

Set. II. — The second set, series E, F and G, was planned differently in order to obtain a uniform relation between the time of the last moult and the date of operation. On January 24, 1907, several hundred isopods were collected and two hundred and fifty normal males measuring between 13–16 mm. selected. These were placed in individual bottles at once. Twenty-four

hours later they were examined and twenty found to have moulted. These twenty were isolated for another twenty-four hours. At the end of the second day, that is, not more than forty-eight hours after their last moult, twelve individuals of about the same size from this twenty were operated upon. Thus a double check was made on the size of the specimens for only those whose length, both before and after the moult was about he same, were retained. Three series were used. In series E the right sixth walking leg was removed to again establish a "standard" series.

In the other two series "added" injury was also inflicted. The removal of right fifth and fourth walking legs constituted this "added" injury in series F and all right walking legs in series G. In the above manner twenty-one individuals were selected and operated upon on the twenty-sixth, nine on the twenty-seventh and eighteen on the twenty-eighth, giving a completed series of sixty individuals, twenty to a series. These series were maintained in the same manner as Set I., save that the water was changed daily. The method of operation was also the same. Two days after their next moult the isopods were killed. By February 11, 1907, twenty-eight had moulted and the set was discontinued.

III. Data.

The length in millimeters of the body, the original leg and the regenerated leg is given for each individual that had regenerated at the close of the experiment. The specific amount of regeneration, which is the per cent. of regeneration in terms of the original leg is given for both sets. The specific rate, which is the specific amount divided by the number of days in the moulting period was obtainable only for Set II.

Explanation of Tables.[1]

Set I. Series A. Right sixth walking leg removed.
Series B. Right 6–5 walking legs removed.
Series C. Right 6–5–4–3 walking legs removed.
Series D. Right 6–5–4–3–2–1 walking legs and cheliped removed.
Set II. Series E. Right sixth walking leg removed.
Serses F. Right 6–5–4 walking legs removed.
Series G. Right 6–5–4–3–2–1 walking legs removed.

[1] Abbreviations: Orig., original; Reg., regenerated; Spec. Amt., specific amount; Spec. Rate, specific rate; 13 +, moulted during last half of thirteenth day.

TABLE I.

SET I. *Series A.*

Cat. No.	Body Length.	Right Sixth Walking Leg.		Spec. Amt.	Moulting Period—Days.	
		Orig.	Reg		1st.	2d.
1	9	3.48	2.52	.72	13	
2	9	4.00	3.19	.80	2	
3	10	3.76	2.82	.76	13	
4	10	3.57	2.48	.69	12	
5	10	4.00	2.71	.67	13	
6	10	3.38	2.90	.86	13+	
7	10	3.76	2.52	.67	7	11
8	10	4.33	2.81	.65	13	
9	11	3.38	1.81	.54	12	
10	11	5.33	2.38	.45	13	
11	12	4.66	3.19	.69	13+	
Av.	10.1			.68		

Series B.

1	10	4.14	2.86	.69	13	
2	10	3.43	2.76	.80	11	
3	10	3.76	2.71	.72	9	
4	11	3.43	2.76	.80	1	8
5	11	4.29	2.19	.51	13+	
6	11	3.67	2.38	.68	8	
7	11	3.52	3.29	.93	4	
8	11	4.05	2.57	.63	13+	
9	11	3.19	2.57	.81	8	
10	11	4.38	2.76	.63	13	
11	12	3.67	3.43	.93	13	
Av.	10.8			.739		

TABLE II.

SET I. *Series C.*

Cat. No.	Body Length.	Right Sixth Walking Leg.		Spec. Amt.	Moulting Period—Days.	
		Orig.	Reg.		1st.	2d.
1	10	4.48	2.82	.63	13	
2	10	3.62	2.86	.79	7	13+
3	10	3.52	2.05	.58	11	
4	11	4.05	2.57	.63	2	13+
5	11	4.29	2.81	.65	13+	
6	11	4.14	2.86	.69	12	
7	12	4.00	3.10	.775	3	10
8	12	4.43	2.95	.86	9	
9	12	4.48	2.43	.54	10	
10	13	4.29	2.82	.88	2	13
Av.	11.2			.703		

Series D.

1	9	3.38	2.33	.66	10	
2	10	3.62	2.57	.71	13	
3	10	3.95	2.76	.69	3	
4	10	3.29	2.52	.77	12	
5	10	3.86	1.91	.49	7	
6	11	4.10	3.33	.81	8	
7	11	3.90	2.71	.72	7	13+
8	11	4.19	2.90	.69	12	
9	12	4.19	3.19	.76	12	
10	12	4.61	3.19	.67	11	
11	13	4.24	1.91	.45	7	
12	13	5.23	2.90	.55	13	
Av.	11.0			.664		

TABLE III.

SET II. *Series E.*

Cat. No.	Body Length.	Right Sixth Walking Leg.		Spec. Amt.	Moulting Period.	Spec. Rate.
		Orig.	Reg.			
1	15	7.25	2.90	.40	12	.033
2	14	5.86	2.38	.41	10	.041
3	14	5.76	2.71	.48	13	.037
4	13	6.00	2.57	.43	14	.031
5	13	5.76	2.71	.48	13	.037
Av.	13.8			.441	12.4	.036

Series F.

1	16	7.19	3.10	.43	17	.025
2	15	7.00	2.95	.42	16	.026
3	15	7.00	3.14	.45	15	.030
4	14	5.67	3.48	.61	14	.044
5	14	6.95	3.00	.43	16	.027
6	14	6.48	3.00	.46	16	.029
7	13	6.14	3.10	.50	17	.029
8	13	5.38	2.43	.45	13	.035
9	13	5.57	2.14	.38	16	.024
Av.	14			.48	15.5	.030

Series G.

1	16	6.48	3.10	.48	15	.032
2	15	7.00	2.57	.37	12	.031
3	15	6.67	2.71	.41	13	.031
4	15	6.24	2.95	.47	13	.036
5	14	6.90	3.14	.46	17	.027
6	14	6.00	2.62	.44	15	.029
7	14	5.33	2.33	.44	16	.028
8	14	5.86	2.67	.46	14	.033
9	14	5.05	2.76	.55	13	.042
10	14	4.95	2.90	.59	15	.045
11	14	6.48	3.10	.45	13	.035
12	14	6.67	2.90	.44	16	.028
13	14	6.43	2.95	.46	13	.044
14	14	4.76	2.29	.48	10	.048
Av.	14.3			.463	13.5	.035

IV. Results.

1. *Specific Amount of Regeneration.*

Set. I. A — .67, B — .74, C — .70, D — .66.

Starting with the standard series A the specific amount of regeneration increases in series B and decreases in series C and D. Both B and C are greater than A, while D is less than A. These values show two important facts: (1) that there is an optimum degree of injury and (2) that there is a limit to the amount of added injury that may be inflicted and the resultant regeneration still be greater than that following the standard injury. This limit of added injury is between C and D.

Set. II. E — .44, F — .48, G — .46.

The specific amounts of Set II. follow precisely the same rule of arrangement as those of Set. I. There is a rise and a fall in the amount of regeneration. However no series of Set. II. is below standard and as a result the limit of added injury does not occur. Since the injury inflicted in series A and E was the same, there being so few individuals in series E it was thought advisable to obtain the values of Set II. in terms of Set I. Accordingly a coefficient (1.542) was established by dividing the specific amount of series A by that of series E. The specific amounts of Set II. were then multiplied by this and the following table made by placing the values of both sets in the order of amount of injury.

A and E — .68, B — .74, F — .74, C — .70, G — .70, D — .66.

In this table of the combined values there is the same plan of increase and decrease in the amount of regeneration as has been noted in both sets. It is a rise at the first followed by a steady decline. The limit of added injury and the optimum are both present.

Considering these tables three things are evident:

1. The amount of regeneration increases directly with the amount of injury until an optimum has been attained.

2. Beyond the optimum added injury still gives an amount of regeneration greater than that of the standard injury up to a limit.

3. Beyond this limit regeneration is less than the standard.

2. *Specific Rate.*

The specific rates for Set I. were not obtainable as the time and not the moult was constant. For Set II. the specific rates were E — .036, F — .030, G — .035. Nothing very certain can be said as to the value of these figures, however, as specific rate may be an unreliable quantity. The greatest source of error in computing it is the moulting period. This could easily be influenced either by (1) the shock of the operation, or (2) by the asymmetrical condition produced by the loss of appendages. The effect of neither was determined, yet because of their existence as possible factors in the rate of moulting the exact worth of the specific rate is not known.

V. Conclusion.

From the data collected it seems probable that an increase in the amount of injury produces an increase in the amount of regeneration until a certain limit is reached. This limit of added injury is probably constant for the species.

As a point of interest it may be noted that the individuals collected in October regenerated about fifty per cent. more in a given time than those collected in January. It seems that the season of the year may have some influence upon regeneration.

VI. Summary.

1. Each leg of *Mancasellus* possesses a breaking joint at the coxal-thoracic articulation.

2. The season of the year may influence the ability to regenerate lost parts.

3. Increased injury increases the amount of regeneration in *Mancasellus* until the optimum is reached. From this it decreases to a limit beyond which the amount of regeneration is less than that of the standard.

4. The optimum seems to be low for *Mancasellus*.

5. The limit of added injury is relatively high.

Acknowledgment.

This work was undertaken and carried out at the suggestion of Dr. Charles Zeleny. I thank him for his willing assistance.

THE SPERMATOGENESIS OF PANDARUS SINUATUS SAY.

J. F. McCLENDON.

The spermatogenesis of the parasitic copepods of the Woods Hole region was included as a preliminary note in a previous paper of mine (McClendon, '06), *Læmargus muricatus* Kröyer being taken as an example. The spermatogenesis of these forms is more nearly identical than the oögenesis. In fact, the greatest difference is in the number and size of the cells in the testes. The advantage in studying *Læmargus* was the greater size and greater number of cells in the testes, but the difficulty of obtaining living material of this genus led to the substitution of *Pandarus*.

The material for the present paper was procured at Woods Hole last summer. The testes with more or less adjacent tissue were removed and fixed in Flemming's stronger fluid. Paraffine sections, 3 to 7 microns in thickness, were cut, and stained in various ways. Iron hæmatoxylin, Delafield's hæmatoxylin, saffranin, orange G, and various combinations were found valuable. Not a great deal of attention was paid to the exact form in which the cytoplasm and nuclear sap was coagulated by the fixation, but attention was directed chiefly to the chromatin of the nucleus and to those remarkable bodies described in the previous paper under the name of nutritive spheres.

The spermatogonia (Fig. 1) are nearly isodiametrical cells with large spheroid nuclei. Each nucleus contains during the rest stage two or more nucleoli (plasmosomes) and a reticulum in which chromatin granules are imbedded. During mitosis sixteen chromosomes are formed and divided (Figs. 2 and 3), one half of each chromosome going to each of the two daughter cells.

The primary spermatocytes when first formed (Fig. 4) are similar to the spermatogonia except for size. The nucleoli never grow to the size they reach in the spermatogonia, probably because the prophase of mitosis begins early in the growth of the cell and the nucleoli begin to dissolve before they have had time

to grow large. The chromosomes in the early prophase (Fig. 5) are thread-like and do not show a longitudinal split as is the case in the primary oöcytes of this species. It may be that the longitudinal split is present but cannot be seen on account of the smaller size of the chromosomes, or the splitting may occur after the synapsis, at which time a division is indicated by a constriction at right angles to the plane dividing the chromosomes of each pair (see below). Another interpretation is that in the primary oöcytes the "split" represents the division between adjacent chromosomes and is therefore after the synapsis. The chromosomes are at first sixteen in number, but as they become denser some appear to be joined end to end (Fig. 6), and by this time the nucleoli have entirely dissolved. The chromosomes collect together in a dense mass so that only their ends sticking out can be distinguished separately (Fig. 7), and after the elements of this mass separate they are seen to be eight double chromosomes united end to end (Fig. 8). The chromosomes now shorten, at the same time becoming thicker (Fig. 9), and soon a second constriction transforms each double chromosome into a tetrad (Fig. 10). Each tetrad continues to shorten until the width is as great as the length (Fig. 11). The nuclear wall dissolves and the spindle is now formed (Figs. 12, 13, 14). In the equatorial plate seven tetrads are arranged in a circle and the eighth lies in the center. It is impossible to observe whether the division is reducing or not, owing to the shape of the tetrads.

The second spermatocytic division follows immediately after the first. Each of the eight diads is divided equally between the two daughter cells (Figs. 15, 16). In the spermatids thus formed the chromosomes swell up and fuse to form nuclei, and the spermatid (Fig. 18) resembles the primary spermatocyte save for the reduction in the size and the absence of nucleoli.

By the methods used no distinction could be made out between the spermatids when first formed, but they develop into structures that show as great differences as exist between the products of the testes of any species of animals that has come to my attention. Many spermatids degenerate and appear to be absorbed as food by those remaining. Some of them elongate (Figs. 19 and 20) and begin to develop into spermatozoa. Whereas the spermatids

when first formed are in groups of fours, as they elongate they collect into larger groups. The cell boundary becomes granular (Fig. 21) and the cytoplasm of adjacent spermatids fuses. Only part of this cytoplasm goes into the formation of the spermatozoa and the remainder forms a mass in the center of the group. When the spermatozoön is fully formed no distinction can be made between the cytoplasmic and nuclear parts of it in fixed preparations (Fig. 22), but it appears as a homogeneous chromatic thread tapering at each end. This spermatozoön is similar to those of barnacles and some other crustacea, and is non-motile in sea water, though it is supposed, like other crustacean spermatozoa to be stimulated to locomotion by the fluid in the ducts of the receptaculum semenis.

In some spermatids the chromatin collects into apparently homogeneous masses close to the nuclear membrane, and the nucleus grows at the expense of the cytoplasm (Fig. 23). This process continues until the cytoplasm is represented by merely a thin granular layer surrounding the distended nucleus (Fig. 24) and finally disappears entirely (Fig. 25). The nuclear sap, which is at first a thin fluid gradually becomes denser until it appears homogeneous on fixation and takes plasma stains. The time at which it acquires this power of taking stains is not sharply marked off, as it appears gradually and as it is determined by the duration of the staining and destaining process, but after all the cytoplasm has disappeared the interior of the "nucleus" is easily stained. Soon the nuclear membrane disappears and the chromatin remains adhering to the surface of the sphere of material that filled cavity of the nucleus (Fig. 26), and which was designated by the name nutritive-sphere in my former paper. The spermatozoa are often arranged with one end against one of these spheres, in a mammer similar to that in which the spermatozoa of many animals are related to the nurse cells.

When the products of the testes pass through the vasa deferentia and enter the spermatophores, the nutritive spheres form a layer next to the wall of the spermatophore, and the chromatin, which had separated as globules, forms a layer inside of the layer of spheres. Lastly the spermatozoa arrange themselves more or less radially, that is, with their ends abutting against the layer of

chromatin globules. Some chromatin globules are found in the spaces between the spheres and become pressed out of shape by a pressure that transforms the nutritive spheres into polyhedrons. In spermatophores which are attached to the female, the substance of this nutritive layer is often found to have disappeared, leaving a structure resembling thin evacuated cell walls.

It would be interesting to know the chemical composition of the nutritive spheres. Heider ('79) probably supposed them to be mucilaginous, as he called them "austreibestoff." In some mosses there is found a mucilaginous substance in the antheridia, which swells in the presence of water and forces the spermatozoa out. A similar function was attributed to these spheres in Copepods by Heider, but I have found no evidence that such is the case. When the spermatophores are attached to the female, direct communication is formed with the receptaculum semenis, and there is no reason to believe that the spermatozoa could not enter the receptaculum by their own efforts. If there is a secretion in the receptaculum that would stimulate the spermatozoa into movement, it would diffuse into the spermatophore and be effective there. I have tried staining reactions on these spheres but can say only that they have a less affinity for plasma stains than the yolk spherules of most eggs, and that they are not of a fatty nature.

The position of the spermatozoa in relation to them and the fact that their substance disappears finally, led me to assume that they furnished nourishment for the spermatozoa. It has long been held that the nucleus influenced the assimilation in the cell, but so far as I know, the idea that the nucleus could form a store-house for the nourishment of other cells is new. I hesitated in putting forward such a view in my previous paper, hoping to discover a cytoplasmic origin of these spheres, but such seems not to be the case. In *Peripatus* the spermatozoa appear to draw nourishment from the nuclei of degenerated spermatids, but it is probable that such is the case in any testis in which cell degeneration occurs. In *Pelomyxa*, Goldschmidt ('06) describes the formation of "Glanzkörper" of the plasmosomes extruded from the nucleus. These "Glanzkörper" are supposed to be glycogen. In the method by which my sections were prepared, glycogen, if present, would probably be entirely washed out.

In the insect, *Ulula hyalina* (McClendon, '02), some of the eggs become modified to serve as protection to the other eggs. These abortive eggs or "repagula" develop in different ovarian tubules from the other eggs, and may receive different nourishment, but are of interest as modified sexual elements.

For comparison, each bundle of spermatids in *Pandarus* may be said to be provided with a cytoplasmic cytophore in the middle of the bundle and a greatly modified unicellular cytophore at the end of the bundle. Cytophores and basal cells are differentiated (visibly) after the second spermatocytic division (Korscheldt & Heider) in invertebrates. Nurse cells in ovaries are differentiated at an earlier stage (Marshall, '07).

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Explanation of Plate I.

All the figures were drawn with Abbe Camera, Zeiss apochromat objective 2 mm., compensating ocular 12. They represent optical sections of varying thickness, of elements of the testes of *Pandarus sinuatus* Say.

Fig. 1. Spermatogonium. The black spheres are nucleoli.

Fig. 2. Anaphase of the last spermatogonial mitosis, seven of the divided chromosomes are shown.

Fig. 3. Telophase of the last spermatogonial mitosis.

Fig. 4. Resting stage of primary spermatocyte. The two black spheres are nucleoli.

Fig. 5. Presynapsis stage of the primary spermatocyte. The chromosomes are in the form of threads and are sixteen in number, but not all are shown in the figure.

Fig. 6. Commencement of synapsis. The chromosomes are denser than in the preceding figure.

Fig. 7. Synapsis stage. The chromosomes are so close together that they cannot be counted.

Fig. 8. Post-synapsis stage. The chromosomes have paired to form eight bivalent elements.

Fig. 9. Prophase of first spermatocytic mitosis. The chromosomes have shortened.

Fig. 10. Later prophase. The bivalent chromosomes are transformed into tetrads by a longitudinal furrow.

Fig. 11. Late prophase. The tetrads have become still more shortened.

Fig. 12. Metaphase of first spermatocytic mitosis.

Fig. 13. Equatorial plate of first spermatocytic mitosis. The eight tetrads are shown.

Fig. 14. Telophase of first spermatocytic mitosis.

Fig. 15. Metaphase of second spermatocytic mitosis.

Fig. 16. Telophase of second spermatocytic mitosis.

Fig. 17. Late telophase of same.

Fig. 18. Spermatid.

Figs. 19–22. Stages in elongation of the spermatid to form the spermatozoön.

In Fig. 21 some of the cytoplasm is being lost and the cell boundary is granular.

In Fig. 22, which represents the spermatozoön, the elongated nucleus and its thin covering of cytoplasm cannot be separately distinguished.

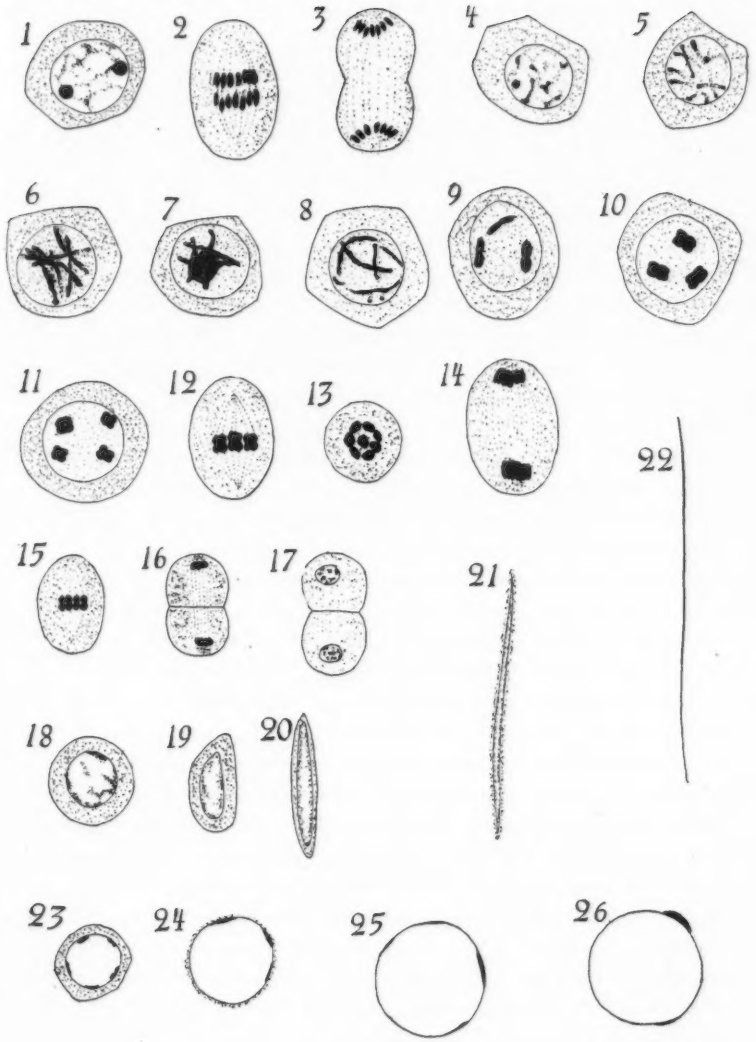
Figs. 23–26 represent stages in the formation of a nutritive sphere from a spermatid.

In Fig. 23 the cytoplasm has decreased in amount and the chromatin has collected into lumps at the periphery of the nucleus.

In Fig. 24 the cytoplasm has disappeared save for a thin granular layer, and the nucleus is distended by the nutritive sphere.

In Fig. 25 the nutritive sphere has increased in size and become denser, while the cytoplasm has entirely disappeared.

In Fig. 26 the nuclear wall has disappeared and the chromatin forms lumps on the surface of the nutritive sphere.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THE ORDER OF APPEARANCE OF THE ANTERIOR SOMITES IN THE CHICK.[1]

J. THOS. PATTERSON.

A. Introduction.

The statement that in the chick, somites arise in front of the first somite formed in the series has been widely accepted by embryologists. This view, nevertheless, is not in accord with our knowledge concerning the early development of birds, for it is well known that differentiation usually begins at the anterior end and progresses posteriorly.

Although workers in this field agree that somites arise anterior to the one first formed, yet they differ as to the exact number. Thus Balfour ('85) states that there is one, while His ('68) and von Baer ('28) have estimated it at two. Kupffer and Benecke ('79) would lead one to believe that there were at least three or four. So far as I am aware the latest work done to determine this number is by Miss Platt ('89), who concludes from a study of sections that there are two, or, to be more exact, one and a half.

From the results of certain experiments, made in connection with an experimental study of the early development of the pigeon, the writer was led to believe that *no* somites were formed in front of the first mesodermic cleft, except, of course, the so-called rudimentary or incomplete anterior cephalic somite. At the suggestion of Professor Lillie I have performed a number of experiments to test the validity of this view. These experiments, in connection with others, were conducted on a farm in Ohio, where I had at my disposal the eggs from fifty laying hens. It was possible, therefore, to collect and incubate the eggs hourly.

It gives me pleasure here to express my thanks to Professor Lillie for his kindness in sending all the necessary equipment for this work from these laboratories, and for his valuable criticisms.

[1] Unless otherwise indicated, the word somite will be used throughout this work to mean protovertebra.

B. Methods.

For opening and sealing the egg I have in the main employed the method first used by Miss Peebles ('98). By the aid of a fine file a small window is made in the shell just above the blastoderm. The operation is then performed and the opening closed with a slightly larger piece of shell (with membrane still attached) from the corresponding part of a fresh egg.

Although I have used very fine glass pins in some of the work, yet I have found the electric needle by far the better means for making the injury. These needles (No. 12 sewing needles, ground as fine as possible on a water stone) were connected with two dry battery cells.[1] Then, by the aid of a binocular, using a combination of lenses giving a magnification of 12.6 diameters, one needle is placed at the desired point and the other is touched to the albumen for a second or two. The opening is then closed in the manner stated above. The whole procedure from the opening to the closing of the egg need not take over a minute.

If no further precautions were necessary the experimental work would be a simple process, but the difficulties that attend experimental studies on the bird's egg are many. Perhaps there is none so perplexing as that of preventing infection. Previous workers have realized this fact. Some writers have reported a loss of embryos, due to mould or bacteria, reaching as high as 80 per cent.

The mere heating of the instruments is not sufficient in itself to prevent infection. However, I find that if one uses a .1 per cent. solution of bichloride of mercury previous to heating, this difficulty is practically overcome. The table upon which the operation is performed, the hands, the instruments, in fact, every thing connected with the operation must be thoroughly washed in this solution. With a cloth moistened in the sublimate I also wipe off the shell where the window is to be made, otherwise small fragments of the shell falling upon the albumen will be a frequent source of infection.

[1] The Cleveland Dry Battery Cells were used. Each cell has a pressure of about 1.3 volts.

[2] All the drawings with which this paper is illustrated were made by the aid of the Abbe camera.

After closing the opening I place over it a piece of sterilized cotton about 5 cm. square and holding down the edges of the cotton on the sides of the shell, slowly revolve the egg until the closed window is on the lower side. It is then placed in a watch-glass and incubated the desired period of time. In addition to holding the piece of shell in place, the cotton prevents the egg from rolling and facilitates handling.

Inverting the egg serves a double purpose. In the first place, no matter how careful one may be a certain number of eggs are sure to become infected by germs falling on the albumen from the air. Now, when the egg is revolved, the yolk turns until the blastoderm is uppermost and hence removed as far as possible from the region of possible infection, which spreads too slowly to reach the blastoderm and interfere with the development of the embryo, especially if the egg is incubated but a few hours. In the second place, by revolving the egg the blastoderm is brought into an environment almost, if not entirely, normal. Mitrophanow ('97) has shown that varnishing the shell above the blastoderm retards development by limiting the supply of oxygen, and produces abnormalities. A similar effect is undoubtedly produced by placing an extra piece of shell above the embryo and sealing down its edges with strips of membrane — a method used by some workers. Any abnormalities thus produced are to be avoided, because they complicate the correct interpretation of one's experimenal results.

In order to test whether inverting the egg brings the blastoderm into a normal environment, some eggs were thus turned, while others were allowed to remain with the covered window uppermost, or turned but slightly to one side. In general, the latter were delayed from two to four hours, while the former developed equally with the controls.

It may seem that the above precautions are a bit tedious and unnecessary, but one is fully repaid for the trouble thus taken, as shown by the following statistics. During the period in which this series of experiments was carried on over 400 operations were made and but five eggs were infected, or less than 2 per cent., and during the present year about 100 operations have been performed with not a single case of infection noted. Of

this entire number between 80 and 90 per cent. of the eggs have given definite results.

C. Experiments.

If only the rudimentary somite arises in front of the first mesodermic cleft, an injury made just anterior to this cleft ought to destroy, at least partially, this incomplete somite; but if, in addition, complete somites are formed anterior to this cleft, the injury ought to appear later in that one of the complete somites lying just in front of the cleft.

In performing such an operation there are two sources of difficulty. In the first place, owing to the individual variation in the early development of eggs, it is very difficult to hit upon the exact time when but one cleft is present. This difficulty can be met by opening the egg one to two hours before the cleft ordinarily appears and temporarily sealing the opening, so that it may be reopened and examined from time to time until the cleft appears. It was found that the average time of appearance of this cleft is between 21 and 22 hours. In the second place, it is almost impossible in a large number of embryos, to see the cleft so that one may be sure of the operation. This is due to the fact that the yolk is often a very pale yellow, and the white embryo cannot be seen. However, in about 20 per cent. of the eggs the yolk is a deep yellow, and against this background the embryo stands out in perfect contrast, and one can be absolutely certain of one's operation. In this work only the latter kind of eggs was used.

Experiment I.

The operation was performed with an electric needle after the egg had been incubated 22 hours at a temperature of 37–39° C. Fig. 1 illustrates the place of injury (Fig. 1, *O*) and the condition of the embryo at the time of the operation. It will be noted that the first cleft lies just anterior to the fore-end of the primitive streak and meets the main axis of the embryo at an oblique angle.

After the operation the egg was incubated ten hours. The embryo was killed in picrosulphuric-acetic acid, stained in Conklin's picro-hæmatoxylin, and mounted in xylol-balsam.

Fig. 2 shows the result of the operation. On the right side the rudimentary somite is greatly disturbed (Fig. 2, *O*) and the

neural tube is slightly injured. Aside from this the embryo is normal in every way. The eighth pair of somites is just being cut off and the heart is forming.

A sagittal section through the somites of this embryo confirms

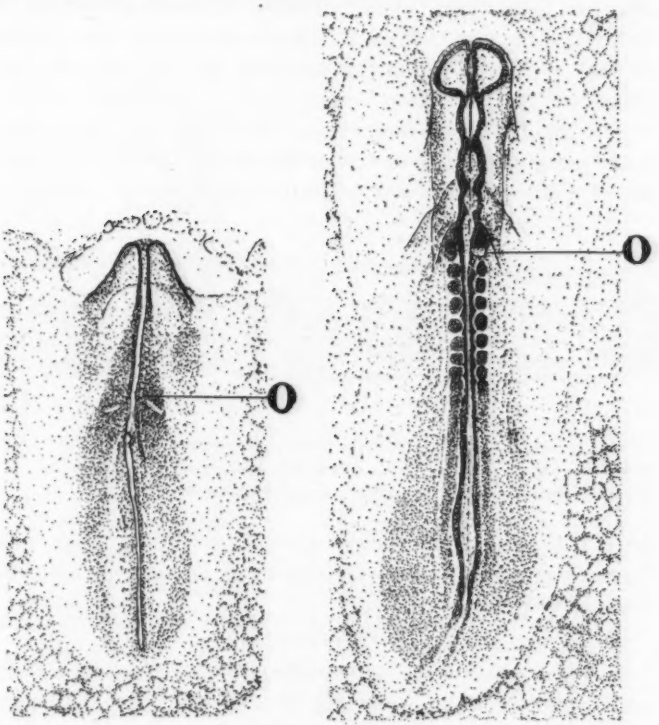


FIG. 1. Twenty-one hours old. It shows the first pair of clefts, which meet the main axis of the embryo at an oblique angle. The place of injury is shown at *O*. × 20.

FIG. 2. Twenty-two hours old when operated on, and then incubated ten hours. It shows eight pairs of somites, and the injury in the right anterior somite at *O*. × 20.

what is seen in surface view. The rudimentary somite is greatly injured (Fig. 3, *x*) and its characteristic enlargement (see Figs. 10–12) is not present.

EXPERIMENT II.

The conditions under which the operation was performed and the subsequent handling of the embryo were the same as in the preceding experiment. Instead of using the electric needle, a very fine glass "pin" was substituted. This pin was placed just in front of the first cleft on the left side at a stage corresponding to that of Fig. 1. After the operation the egg was incubated twenty and one half hours longer.

The result of the operation is shown in Fig. 4. The needle is found in the incomplete somite on the left side (Fig. 4, *O*). There are 16 pairs of somites and the heart is well formed. It was im-

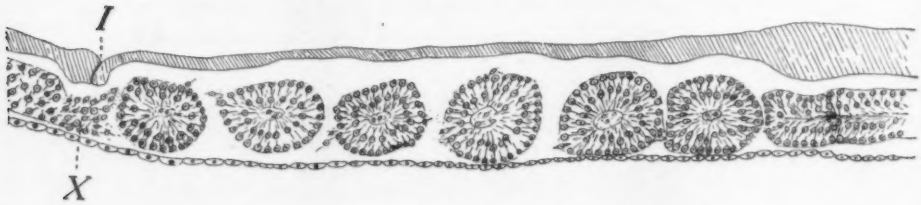


FIG. 3. Sagittal section through the somites on the right side of the embryo represented in Fig. 2. It shows at *X* the destroyed incomplete somite and at *I* the place where the needle has broken through the ectoderm. × 157.

possible to section this embryo on account of the glass pin, but the surface view is so clear that there can be no doubt as to the position of the pin.

EXPERIMENT III.

In order to show that the mesoderm lying between the injury and the cleft (between *r* and *a*, Fig. 6) does not increase subsequent to the operation and antecedent to the examination of the result and give rise to one or more somites, the injury was made so as to destroy the mesoderm spanning the first cleft (Fig. 6, *a*).

The result of such an experiment is well illustrated in Fig. 5. This embryo was treated in exactly the same manner as the one represented in Fig. 2. In addition to destroying the posterior edge of the incomplete somite, the injury extends over a portion of the cleft (Fig. 5, *O*). In this, as in Fig. 2, sections confirm what is seen in surface view.

The above are only a few types out of about seventy-five ex-

periments performed to throw light on the order of development of the somites. In all cases the results support the view that only the incomplete somite arises anterior to the first mesodermic cleft. In the cases cited, the oldest embryo was carried to the sixteen-somite stage, but other embryos were allowed to de-

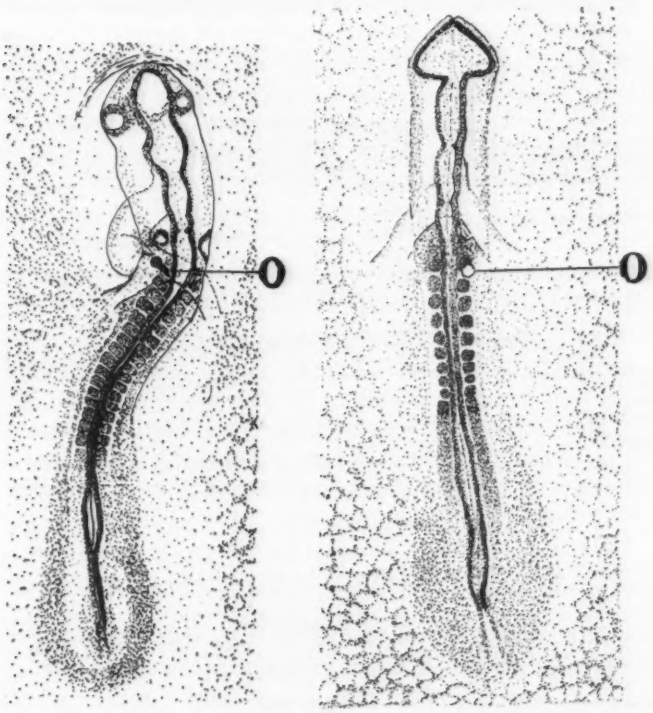


FIG. 4. Twenty-two hours old when operated on, and then incubated twenty and one half hours. There are sixteen pairs of somites. The glass pin is located in the incomplete somite on the left side, at *O*. × 20.

FIG. 5. Twenty-two hours old when operated on, and then incubated ten hours. The injury is shown at *O*, and ten pairs of somites are present. × 20.

velop until twenty or twenty-five somites appeared, with the injury still found in the rudimentary somite. In another series the injury was made just posterior to the first cleft. In such cases the first complete somite was either greatly disturbed or entirely destroyed.

D. Study of Sagittal Sections.

The results of the above series of experiments are in themselves sufficient to prove the position taken in this paper. But inasmuch as some writers have drawn their conclusions from a study of sections, it seems advisable to introduce a few figures to show that sections support the above contention. By far the

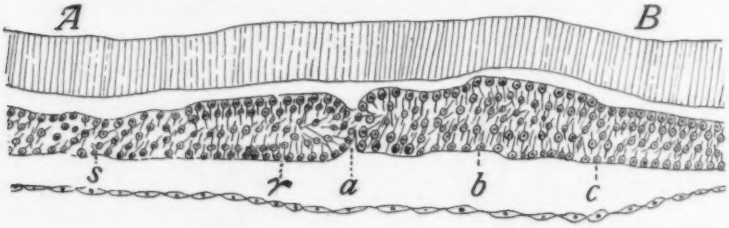


Fig. 6. Shows the first mesodermic cleft, and there are also indications of the second and third clefts. *r*, beginning of the rudimentary somite. × 247.

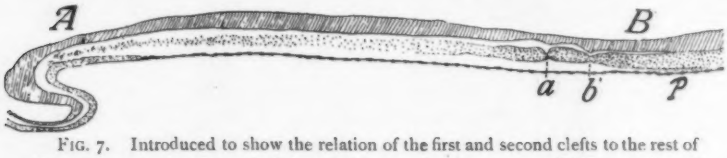


Fig. 7. Introduced to show the relation of the first and second clefts to the rest of the embryo. *p*, anterior end of the primitive streak; *b*–*p*, region of differentiation of the embryo. × 68.

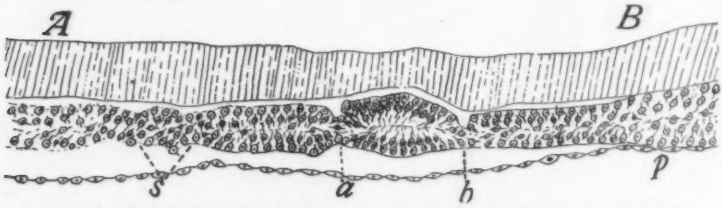


Fig. 8. Enlarged portion of the opposite side of the embryo represented in Fig. 7. *s*, shallow depression. × 247.

Figs. 6–8. In these, as in the remaining figures of this paper, *A* is anterior and *B* posterior end, and *a*, *b*, *c*, etc., are respectively the first, second, third, etc., clefts.

most critical study made on sections is by Miss Platt, whose view is well summed up in her conclusion, in which she says: "My conclusions are, therefore, that the first break in the mesoderm occurs anterior to the first protovertebra, and that two protovertebræ (or, more correctly, one and a half) are slowly formed

nterior to the first mesodermic cleft, in the time occupied by the formation of six or seven protovertebræ posterior to that cleft."[1]

The first indication of somites is a depression on the dorsal surface of the mesoderm lying at the sides of the notochord, just anterior to the fore-end of the primitive streak. This is soon followed by a corresponding indentation on the under side (Figs. 6 and 7, *a*). Between the cleft and the anterior end of the primitive streak, indications of the two succeeding clefts are already present (Fig. 6, *b* and *c*). In front of the cleft, the strip of mesoderm which extends forward into the head gradually thins out anteriorly. At some points this thinning out has progressed more rapidly than at others, giving rise to shallow, transitory depressions (Figs. 6 and 8, *s*), which have been wrongly interpreted as clefts by Miss Platt.

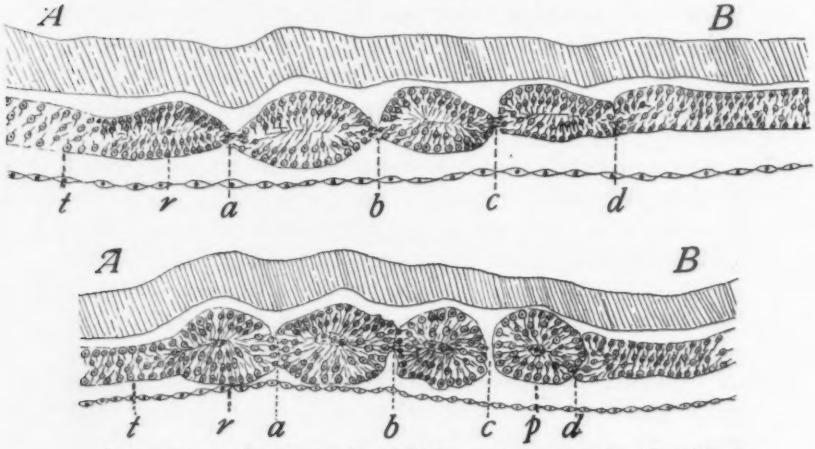
The mesoderm immediately anterior to the first cleft is destined to form the rudimentary somite, and the manner in which this structure arises can be followed with no small degree of certainty. At first the mesoderm is quite uniformly thick (Fig. 9, *t*), but by the time three or four somites are formed its posterior edge has become much enlarged. Apparently this thickening takes place at the expense of the mesoderm just anterior to it (cf. Figs. 9–11, *t*). It should be remembered, however, that this incomplete somite is never so large as the others, really being, as Miss Platt states, only a half-somite. It must be considered as a part of the head mesoderm, from which it never becomes separated (Figs. 9–12, *r*).

Miss Platt used the relative depths of the clefts as a means for determining the priority of somites. After very justly criticising Kupffer for judging either the fourth or fifth somite to be the oldest on account of its size, she says: "I think enough has been said to show that neither the size of the protovertebræ, their relative distance from the primitive streak, nor yet their obliquity to the main axis, is a sufficient ground to warrant a decisive answer to the question in regard to the order of their development,"[2] and I should add, that neither can the depth of the cleft be taken as a criterion for ascertaining seniority. The error into which one

[1] *Loc cit.*, p. 178.
[2] *Loc. cit.*, p. 174

falls by using such an index, becomes apparent on examination of sections such as are represented in Figs. 9 and 10. In Fig. 9, clefts *a*, *b*, and *c* are so nearly alike that it would be impossible to say which is the oldest, judging from their depths.

In Fig. 10 cleft *b* is slightly deeper than *a*, but *c* is still deeper. In such cases, those who accept the relative depths of the clefts as a criterion for drawing conclusions, would be forced to say that two and one half somites arise anterior to the first formed somite, because, as Miss Platt correctly states, "the first cleft lies



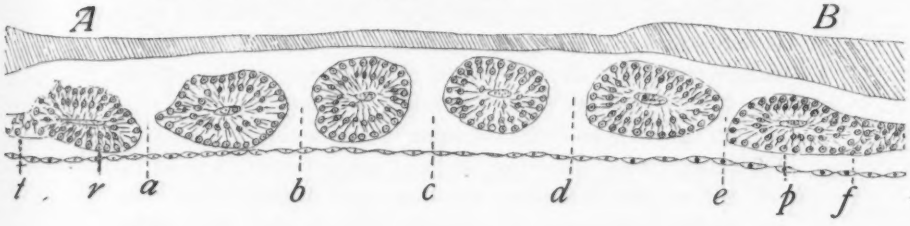
FIGS. 9 and 10. Sections of three and four somites respectively. Both figures show the failure of the first clefts to cut off completely the anterior somites in the beginning. × 157.

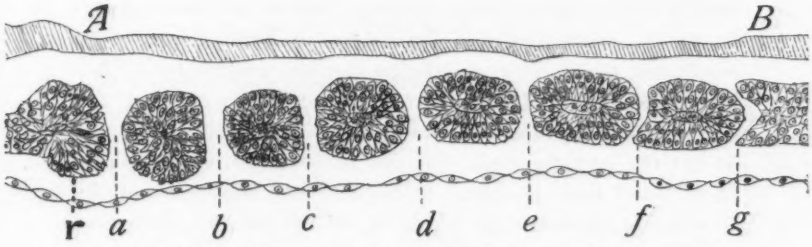
anterior to the first protovertebra, not posterior, as Kupffer and Benecke supposed." [1] In other cases I have observed the fourth cleft to be the deepest.

The condition seen in Fig. 10 is a very common one, and is brought about by the manner in which somites posterior to the first two or three are cut off. In the beginning the first clefts never completely separate their bordering somites, so that the anterior somites often remain connected until the sixth or seventh pair is formed, but posterior to these anterior clefts, succeeding somites are delimited, often before there are any indentations on

[1] *Loc. cit.*, p. 176.

the upper or under surface of the mesoderm (Fig. 11, *f*). As seen in section, the forming somite is at first elongated, with its long axis coinciding with that of the embryo (Fig. 11, *p*). However, it soon rounds up (Fig. 10, *p*), and in so doing becomes separated from the posterior mesoderm (Fig. 12, *g*). The cleft thus made is never vertical, but is so formed that the posterior edge of the last formed somite is in the shape of a wedge, which fits into a corresponding concavity on the anterior edge of the unsegmented mesoderm (Figs. 10–12). That the above process





FIGS. 11 and 12. Sections of six and seven somites respectively. *r*, rudimentary somite. × 157.

of cutting off somites is a rapid one, is shown in Fig. 10, in which it will be noted that the somite is completely formed before there is any indication of one succeeding it.

E. Discussion and Conclusion.

Since Miss Platt's account of the formation of the incomplete somite is not essentially different from that of mine, it follows that we differ only as regards one cleft. According to Miss Platt's view one cleft slowly forms in front of the first one, and hence one complete somite arises anterior to the first formed somite.

I have shown that this author has mistaken the most posterior of certain transitory shallow depressions in the head mesoderm for a cleft. I have also made it clear that the first few (2 or 3) clefts are not completed until six or seven pairs of somites are formed. It seems reasonable to suppose, therefore, that Miss Platt has interpreted the first cleft, during the early stages of development, as a derivative of the most posterior shallow depression. It should be added that this posterior shallow depression persists longer than the others.

Since these shallow transitory depressions are situated at regular intervals, I might suggest that they lend themselves to another interpretation, namely, as vestigial clefts separating the cephalic mesoblastic somites. Notwithstanding the fact that Locy ('95) and his followers minimize the value of myotomes in ascertaining the metamerism of the vertebrate head and use neuromeres as the *sine qua non* for determining primitive segmentation, nevertheless the glimpses one gets of such structures as that cited above should not be overlooked. In fact, if these vestigial clefts are studied in connection with the various conditions seen in the myotomes, the above interpretation becomes evident, for in passing backwards from the anterior end of the embryo one finds that the clefts become more and more pronounced. This is evidenced by (1) the vestigial clefts, (2) the rudimentary somite, whose anterior cleft fails to separate it from the head mesoderm, (3) the slowness of the first clefts in cutting off the anterior protovertebræ, (4) and finally the sharpness and rapidity with which all succeeding protovertebræ are cut off. In other words the influence of the process which has completely obliterated or greatly modified the anterior cephalic somites, gradually becomes weaker in passing posteriorly, and finally ceases altogether.

In regard to the experimental work it seems unnecessary to add to what has already been said. It was noted that an injury made, either with a glass pin or an electric needle, just anterior to the first cleft, appeared upon further incubation, in the rudimentary somite, showing beyond a shadow of doubt that no somite, except the incomplete one, is formed anterior to the first mesodermic cleft. This brings the order of the appearance of somites of the chick into harmony with the general law for the early devel-

opment of the embryo, namely, that differentiation begins at the anterior end and progresses posteriorly.

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UNIVERSITY OF CHICAGO,
April 3, 1907.

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SUGGESTED EXPLANATIONS OF CERTAIN PHENOMENA IN THE LIVES OF ANTS; WITH A METHOD OF TRACING ANTS TO THEIR RESPECTIVE COMMUNITIES.

ADELE M. FIELDE.

A. During summers spent upon Cape Cod and Cape Ann, I have observed a preponderance of queen-pupæ in the nests of myrmicine and camponotine ants in the month of June, and a marked diminution or absence of such pupæ late in summer. This observation has led me to believe that queens may be the issue of eggs deposited in the preceding summer, having passed the winter in the larval stage in company with the hibernating ant-nurses. They would thus receive the earliest attention of these nurses at the beginning of the warm season and would then acquire the size and traits that distinguish them from the workers, the workers being more rapidly developed from eggs deposited during the summer in which they hatch. It is well established that the activities of the ants increase with the temperature[1] up to 85° F. or 30° C. The maximum activity of the nurses, with the more abundant food-supply in summer, should make queen-pupæ more numerous in early autumn than in early summer, were these pupæ the product of eggs deposited during the summer in which the queen-pupæ are discovered. The relative paucity of insect food, the comparative inactivity of the ant-nurses during the spring, and the brevity of the interval between the emergence of the ants from the hibernating place and the discovery of the queen-pupæ, render it fairly certain that these pupæ had attained the larval stage previous to the retirement of the ants into the deep recesses of their nest at the approach of the preceding winter.

[1] "Observations on Ants in their Relation to Temperature and Submergence," A. M. Fielde; BIOLOGICAL BULLETIN, Vol. VII., No. 3, August, 1904, and "Temperature as a Factor in the Development of Ants," A. M. Fielde, BIOLOGICAL BULLETIN, Vol. IX., No. 6, November, 1905.

B. Last summer, on Cape Ann, I kept a colony of formicid ants, that were living in their natural nest under a single stone, abundantly supplied with crushed insects, sugar, and sponge-cake, the supply being protected from rain and renewed at least twice a week. I also arranged annexes to the nest, and these proved so acceptable to the ants that they moved part of their colony into my additions to their residence. At the end of June, when I first observed this nest, there was a great number of queen-pupæ in it but no queen was visible to me. Early in July there were numerous newly hatched queens, and from that time until the eighth of September, when I left Cape Ann, the queens were countless, and there had been no observed swarming from the nest. Somewhat similar observations made by me upon other natural nests have suggested the possibility that the retention of more than one queen by certain species of ants, including *Stenamma fulvum* may result from abundance of nutriment, attainable without excessive labor on the part of the workers.

C. In my formicaries, ants of two species, *Lasius latipes* and *Camponotus herculaneus*, neither of which is a tent-builder, as well as the tent-building ant, *Cremastogaster lineolata*, have at different times protected their young from light by making, during the night, a continuous layer of small pellets of earth on the top of a pane of transparent glass, that I had placed horizontally over a hollow in which the young were assembled. Since all ants habitually withdraw their young from the ultra-violet rays of light, it appears probable that the tent-building ants erect their peculiar structures for the purpose of shielding their young from these rays; and the above recorded observations, on ants of another subfamily, indicate that specific conditions may impel other than tent-building ants to become tent-builders.

D. For correct interpretation of the behavior of ants observation needs be indefinitely prolonged. Sometimes the real animus of one ant toward another is revealed only after weeks or months of continuous association. Some ant-sisters, *Camponotus pennsylvanicus*, reared by me and indisputably the offspring of the same queen-mother, were separated all their lives, the younger in one group, and their elders by a year in another group. The two groups were gradually and cautiously made acquainted with one

another, and the younger sisters were supposed by me to have become reconciled to the progressive odor of their seniors. But after being united in an apparently congenial family group, a senior worker was occasionally killed by a junior, and successive conflicts utterly destroyed the colony after several months.

I have repeatedly observed the gradual dwindling and extinction of apparently healthy ant-groups in which the individuals bore odors not wholly familiar to all the inhabitants. Into an artificial nest of *Camponotus herculaneus*, in which the occupants were all virgin workers, who had never before met a male of their species, I introduced several males. The first impression of an inexperienced observer would probably have been that the workers attacked the males with intent to tear them limb from limb. For some hours the attacks were maintained; but the males remained unscathed and, without even a rent in their delicate wings, continued for weeks in close companionship with the workers. It was attraction, not antipathy, that dictated the violent behavior of the workers.

The presence of young; the completeness of the establishment of the nest-aura; the domestic conditions in general; the familiarity of the ants with their immediate environment; the incurred odor; the inherited odor; the progressive odor; the specific odor; the sensitivity of the ants to a preponderating odor and their encouragement or discouragement therefrom; the aptness of ants to concentrate attention upon an immediate interest and to become temporarily oblivious to other matters; and the associative memory maintained by every ant concerning its previous experiences, are all factors which need be weighed when determining the causes of the behavior of ants.

E. When making inquiry of the ants concerning their sense of hearing in the summer of 1903, I deprived the ants of portions of their bodies and found that the excision of certain parts uniformly affected the direction of the movement of the ants when they were startled.[1] Normal queens (of *Stenamma fulvum*) moved either forward, backward or sidewise, while queens de-

[1] "The Reactions of Ants to Material Vibrations," by Adele M. Fielde and George H. Parker, *Proceedings of the Academy of Natural Sciences of Philadelphia*, November, 1904, p. 646.

prived of both antennæ invariably moved backward or sidewise, never forward, and queens deprived of the abdomen always moved forward or sidewise. Since the publication of that paper, it has appeared to me probable that the uniform differences in the direction of movement coincident with uniform maiming of the ants, might be explained by the change of the location of the center of gravity within the body of the ant. Change of the location of the center of gravity in the body of a queen upon the loss of her wings after mating may also explain certain changes observable at that period in her characteristic behavior. The retiring tendency of the queen after deälation may be due to the change in her center of gravity.

F. Dr. H. A. Parr, of New York, has mentioned to me an unpublished method used by him in tracking ants to their respective colonies. From a fleck of raw cotton he makes a minute torch-shaped ensign, colors the bluffy end in an anilin dye, and dips the hard-twisted handle into melted sugar. Ants will pick up this flag-like object, hold it by its sweet handle, and carry it homeward. Being very light and flexible, it does not greatly hinder the bearer in her progress through grasses and among stones; the brilliant pennon is easily followed by the eye of the observer; and different ants are distinguished by the different colors that they carry. This device enables the observer to track ants through long distances and to ascertain whether those discovered at a common rendezvous belong to one or to diverse communities.

New York City,
April, 1907.

STUDIES ON THE RELATION BETWEEN AMITOSIS AND MITOSIS.

III. Maturation, Fertilizaton, and Cleavage in Moniezia.

C. M. CHILD.

Since the present paper is concerned primarily with the rôle played by amitosis and mitosis, respectively in the later history of the ovum and the early development of *Moniezia*, the stages of egg-maturation and fertilization are in themselves of secondary importance. It has seemed advisable, however, to include an account of these stages, although it has not been possible to attain certainty on a number of points, *e. g.*, the polarity of the egg before maturation, the direction of division of the chromosomes in maturation, the origin of the cleavage centrosomes, etc. Failure to reach definite conclusions upon these points is due, at least in large measure, as will appear, to the character of the material rather than to insufficient observation. Much time and labor has been expended in the attempt to obtain positive data upon these points but thus far without success.

The cleavage of the egg is considered from a cytological rather than a morphological standpoint, and much that is of importance embryologically is not discussed, as aside from the present purpose. All the figures of maturation, fertilization, and almost all of those of cleavage-stages are from *Moniezia expansa* but no essential differences have been discovered in the two species. The magnification is the same as that used in preceding papers of the series.[1]

I. Maturation of the Egg.

In point of time the entrance of the spermatozoön precedes the process of maturation and the growth of the male pronucleus occurs during the formation of the polar bodies. For the sake

[1] Child, C. M., "Studies on the Relation between Amitosis and Mitosis. I., Development of the Ovaries and Oögenesis in Moniezia." Biol. Bull., Vol. XII., No. 2, 1907. II., Development of the Testes and Spermatogenesis in Moniezia. Biol Bull., Vol. XII., Nos. 3 and 4, 1907.

of clearness, however, the two processes are described separately, the figures being sufficient to show the conditions as regards both maturation and fertilization at different stages.

When the egg leaves the ovary on its way to the uterus it is flattened or irregular in shape, without any visible polar differentiation so far as could be discovered, and contains a large nucleus with very large nucleolus. Within the nucleus all traces of the spireme which appeared at the beginning of the growth period[1] have disappeared and except for the nucleolus the nuclear contents, like those of many other egg-nuclei at this stage do not take nuclear stains.

Fig. 1 (Pl. II.) shows an egg at this stage but with the spermatozoön entering. In this egg two bodies shown below the nucleus are probably the centrosomes of the first maturation spindle, though it was impossible to be certain on this point.

With the entrance of the spermatozoön a vitelline membrane is formed (Fig. 2 et seq., Pl. II.). Chromosomes soon begin to form in the nucleus (Figs. 2 and 3, Pl. II.).

In Fig. 4 (Pl. II.) an early stage of the first maturation spindle is shown. Here the outline of the nucleus is still visible and the spindle appears to be wholly intranuclear. The very large centrosomes at the poles show a distinctly differentiated outer boundary which appears almost like a membrane. One of them in this figure shows two deeply staining granules, the other, none. The appearance and division of these central granules, or centrioles, is seemingly rather irregular as the following figures indicate. In no case has any trace of asters been observed at any stage of maturation, but with the formation of the spindle, the yolk spherules arrange themselves about the equatorial region. This arrangement of yolk spherules indicates that conditions about the poles of the spindles are similar to those in species where distinct asters appear. It is probable that the absence of asters is not due to any fundamental difference in the character of the processes in this case as compared with cases where asters are visible, but rather to the nature of the protoplasm or perhaps to the energy of the processes involved. The achromatic spindle-structures themselves are exceedingly delicate and often only

[1] Child, C. M., Biol. Bull, XII., 2, 1907.

very faintly visible. It is possible that some method of fixation which I have not employed may serve to render these phenomena more clearly visible. In any case, however, it seems improbable that the presence or absence of visible asters can be regarded as of essential importance.

It has not been possible to determine with certainty the number of chromosomes, though it is not far from eight, the most frequent number in the spermatocytes.[1] The arrangement of the chromosomes upon the spindle is very irregular and anything approaching a typical equatorial plate is rarely seen. The chromosomes exhibit the form characteristic of heterotypic divisions, but no conclusions were possible regarding the direction of division. Figs. 5 and 6 (Pl. II.) show other examples of the first maturation spindle. In Fig. 6 the extremely irregular passage of the chromatic material to the two poles is shown. The chromosomes often appear to be more or less broken up into chains of granules, which seem in some cases to become wholly separated from each other. It is impossible, of course, to determine by observation whether the chromosomes maintain their individuality during this process, but cases of this kind certainly do not appear to strengthen the hypothesis of individuality.

In some cases a nucleus with distinct membrane is formed after the first maturation division (Fig. 7, Pl. II.). In other cases, and apparently more frequently, the second polar spindle appears without an intervening resting stage (Fig. 8, Pl. II.).

The first polar body is of large size (Figs. 7 and 8, Pl. II.) and the centrosome is frequently visible beside the nucleus in it (Fig. 8, Pl. II., Figs. 9 and 10, Pl. III.). This centrosome stains more deeply than in earlier stages and still later apparently undergoes condensation to such an extent that it stains as deeply with iron-hæmatoxylin as a mass of chromatin or a yolk granule.

Fig. 8 (Pl. II.) represents an early stage of the second maturation spindle and Figs. 9 and 10 (Pl. III.) later stages. Fig. 10 is one of the very rare cases in which anything like a typical anaphase has been observed. The change in position of the spindle during its development is apparent by comparison of Fig. 8 (Pl. II.) with Fig. 9 (Pl. III.).

[1] Child, C. M., BIOL. BULL., XII., 4, 1907.

The second polar body is, as is usual, smaller than the first (Fig. 11, Pl. III.) both as regards nucleus and cytoplasm. Division has not been observed in either polar body, though in one case what appeared to be an abnormal or abortive mitosis was seen in the first polar body.

Fig. 11 (Pl. III.) shows the reconstitution of the female pronucleus by the fusion of vesicles which doubtless arise from different chromosomes.

II. Fertilization.

The spermatheca opens into the oviduct at a point near the junction of the latter with the ovary. At the time when the eggs pass into the oviduct the spermatheca is distended with great numbers of spermatozoa a few of which are found in the narrow duct connecting spermatheca and oviduct. The spermatozoön meets the egg as it passes along the oviduct past the opening of the spermathecal duct.

Apparently the passage of the eggs through from the ovary to the uterus is periodical for among the large numbers of proglottids sectioned which contained cleavage-stages in the uterus and fully grown unfertilized eggs in the ovary only a very few show eggs in the oviduct. Among these, however, it has been possible to find a few cases showing the entrance of the sperm.

Fig. 1 (Pl. II.) shows the clearest case observed of this stage. It will be recalled[1] that the fully developed spermatozoön is greatly elongated and filiform without any trace of a visibly differentiated head-region, except that its diameter is slightly greater anteriorly than posteriorly.

In all cases where the spermatozoön was found on the egg it was in contact with the surface over more or less of its length, as if adhering to it. Frequently it was wound several times about the egg, the remaining portion hanging free in the oviduct or extending into the spermathecal duct.

In Fig. 1 the course of the spermatozoön body over the surface of the egg is indicated but only a fraction of the length of the spermatozoön is shown. The figure scarcely exaggerates the clearness of the section itself. That portion of the sperm which

[1] Child, Biol. Bull., XII, 4, 1907.

has entered the cytoplasm of the egg forms a deeply staining rounded mass in strong contrast to the remaining portions. Surrounding it is an area of cytoplasm staining less deeply than other portions of the egg — indicated in the figure by the dotted line. The appearance of the head-like structure is all the more remarkable since no trace of anything of the kind is visible before entrance. Evidently the anterior end of the spermatozoön loses its greatly elongated form after it enters the egg. It has been impossible to determine how much of the spermatozoön is involved in this change and also whether the remaining portions, if any, fuse with the cytoplasm or are cast off.

It is impossible to distinguish with certainty the male pronucleus from small yoke-spherules after the "tail" of the spermatozoön disappears. The earliest stages observed with anything like certainty are shown in Figs. 2, 3 and 7 (Pl. II.). By the time the egg reaches the stage of the second polar spindle, however, the male pronucleus can usually be found in some part. In the eggs shown in Figs. 9 and 10 (Pl. III.) it was present in other sections.

By the time maturation is completed the male pronucleus has attained large size and shows a faintly staining reticulum with a nucleolus of large size. Figs. 11–14 (Pl. III.) show the two pronuclei at various stages of approximation to each other.

It has not been possible to obtain the slightest evidence in support of the view that the cleavage centrosomes arise from the spermatozoön. In the early stages of the male pronucleus (Figs. 2, 3 and 7, Pl. II.) no trace of spheres or centrosomes has been observed in connection with it. In a number of cases the two centrosomes have been observed lying near the pronuclei before cleavage (Figs. 12, 13, 14, Pl. III.) but in no case was there the slightest indication that they were more closely associated with one nucleus than with the other.

There can be little doubt from these observations that the peculiar spermatozoa of *Moniezia* actually fertilize the eggs and therefore that they contain nuclear substance in some form or condition, or at least substance capable of giving rise under proper conditions to a nucleus. If future investigation shall establish what seems at least possible from my own observations, viz., that

functional spermatozoa arise from the "spermatid" nuclei, which themselves arise by fragmentation of the spermatocyte-nuclei,[1] we shall be forced to the conclusion that fertilization is possible without the typical process of maturation.

III. Cleavage.

The relations of the two pronuclei at the time of the first cleavage varies considerably in different eggs. In some cases the chromosomes form (Fig. 12, Pl. III.) and the membrane disappears while the pronuclei are still more or less widely separated. In such cases the chromosomes appear in two distinct groups on the two sides of the spindle (Figs. 15 and 16, Pl. IV.). In other cases the pronuclei approach more closely (Fig. 13, Pl. III.) or even undergo more or less complete fusion (Fig. 14, Pl. III.) before the disappearance of the membrane. In such cases the chromosomes may or may not appear in two groups according to the completeness of the fusion. In one case the chromosomes of the first cleavage were observed in two distinct groups within a single nuclear membrane (Fig. 17, Pl. IV.) and in many cases no trace of the paternal and maternal groups was visible (Figs. 18, 19, 20, Pl. IV.).

The position of the first cleavage-spindle varies greatly as is evident from Figs. 15, 16, 18, 19 and 20 (Pl. IV.). Since nuclear division proceeds much more rapidly than cytoplasmic division during early cleavage, and since the polar bodies soon degenerate or are absorbed, it has not been possible to determine whether the plane of the first cleavage is uniform in position, *i. e.*, whether the first cleavage-spindle finally attains a definite typical orientation in the egg. From my observations on cleavage stages I am somewhat inclined to doubt that this is the case, although my data are not conclusive.

There can be no doubt that the first cleavage is usually mitotic but occasionally conditions are found which might readily be regarded as cases of amitosis. Frequently eggs containing only a large single nucleus which is apparently giving rise amitotically to a smaller nucleus are found. None of these cases are figured since it is by no means certain that they are normal phenomena.

[1] Child, C. M., Biol. Bull., XII., 4, 1907.

In many proglottids a certain proportion of the cleaving eggs undergoes degeneration sooner or later, and such eggs usually show irregular nuclear fragmentations. It is therefore possible that the amitotic first cleavage may be an indication of degeneration. Possibly such eggs are not fertilized and are therefore incapable of normal development.

But although the first cleavage is usually or always mitotic, there can be no doubt that amitotic division appears very early in the course of cleavage.

In most cases a number of nuclear divisions occur before cell-boundaries become visible in the egg (Fig. 21, Pl. V.). Cases of mitosis are rarely seen after the first cleavage but amitosis is of frequent occurrence (Figs. 21–26, Pl. V.). It was impossible to determine whether the cleavage exhibited any regularity, for no basis for orientation was discovered. As cleavage proceeds, the egg is gradually divided into blastomeres containing yolk and blastomeres without yolk. In earlier stages the yolk-bearing blastomeres often contain two or more nuclei (Figs. 23, 26, Pl. V.), but in later stages after cytoplasmic cleavage is more advanced they usually contain one relatively large nucleus (Figs. 28, 29, 30, Pl. VI.). In other words as these yolk-bearing blastomeres are gradually reduced in size by successive cleavages the cytoplasmic cleavages keep pace more nearly with the nuclear divisions.

In the yolkless portions of the egg, however, nuclear division continues to be far in advance of cytoplasmic division as far as the cleavage has been followed (Figs. 27, 29, 30, 31, Pl. VI.; 32, Pl. VII.): the consequence is that each blastomere contains several or many nuclei of relatively small size. Evidently the nuclei in these yolkless portions of the egg are dividing much more rapidly than those in the yolk-bearing portions and, as is evident from the figures of Plates V. and VI., amitosis is the typical method of division.

Rarely a case of mitosis is observed: in all the hundreds of eggs in cleavage stages which have been examined, not more than a dozen cases of mitosis have been seen in stages later than the first cleavage. When mitosis occurs it apparently always involves one of the larger nuclei. Mitotic divisions of the small

nuclei in stages like those shown in Plate VI. have never been observed. The smaller nuclei are, without doubt, dividing more rapidly than the larger, and we are probably justified in concluding that amitosis occurs in those regions of the egg where division is most rapid, while mitosis is found, when it occurs at all, among the nuclei which are dividing more slowly.

In Figs. 28 and 30 (Pl. VI.) two cases of mitosis are figured. In these two cases, in fact in every case of mitosis observed during later cleavage, the spindle lies within a blastomere which is bounded on all sides by a distinct membrane. This is particularly well shown in Fig. 30 where the blastomere undergoing mitosis forms a spherical mass with distinct membrane in the midst of the egg-syncytium. This fact, like others mentioned in preceding papers,[1] seems to indicate that conditions in regions where mitosis occurs are widely different from those in which nuclear division is amitotic. Evidently some physical or chemical condition is present in the region about the mitotic spindle in Fig. 30 which determines the formation of a cell-membrane about a certain mass of the cytoplasm. It is not impossible that the membrane may be a coagulation-product resulting from difference in electrical condition of the colloids in the two regions. Suggestions of this nature have been made by various authors, both as regards nuclear membranes and cell-membranes of this character.

As is evident from the figures, the embryos are quite irregular in shape. As cleavage proceeds, however, elongation commonly occurs, but whether this elongation is a mechanical deformation resulting from pressure of the uterine walls as the egg is forced through narrow openings, or whether it is a typical feature of morphogenesis is not certain. Such cases as Fig. 32 (Pl. VII.) in which one end of the embryo is drawn out into a sharp point seem to indicate mechanical deformation.

Even in these later stages of cleavage it has not been possible thus far to discover any certain basis for orientation of the embryo. In many cases one large yolk-bearing blastomere is found at each end of the elongated embryo (Fig. 27, Pl. VI.). In other cases only one such blastomere appears (Figs. 30, 31, Pl. VI.). My

[1] Child, Biol. Bull., XII., 2, 3, 4, 1907.

observations certainly indicate, though they are not sufficient to prove positively, that cleavage is indeterminate and exceedingly irregular.

My material thus far has not included stages of development later than those shown in Plate VI. I hope in future, however, to obtain later stages and to continue the study of the rôle played by amitosis and mitosis in the later development.

In Figs. 33–40 (Pl. VII.) several single blastomeres and nuclei from various stages of cleavage are figured as showing particularly clear and convincing cases of amitosis.

IV. General Observations.

In this section a few observations of general biological interest though not connected with the chief purpose of the paper are briefly given. As was pointed out in the first paper of this series[1] the eggs in those portions of the ovary nearest the opening of the oviduct begin and complete their growth earlier than the others. Proceeding from this region toward the tips of the ovarian follicles, we find that oögenesis is slightly later at each successive level. Thus the eggs nearest the oviduct are the earliest, while those at the tips of the follicles are the latest to attain full growth.

There can be no doubt that the passage of ova from the ovary to the uterus is periodical and not of continual occurrence, for in most proglottids in which embryos are present in the uterus and immature eggs in the ovary, no eggs are found in the oviduct. In such proglottids nothing later than late maturation stages or cleavage stages is found in the uterus. Occasionally, however, a proglottid is found with eggs in the oviduct, and such cases show the entrance of the spermatozoa in those eggs in the oviduct and usually earlier maturation stages in the eggs in the lateral region of the uterus.

The egg usually reaches the lateral portions of the uterus before the appearance of the first maturation spindle. In the uterus, maturation stages and early cleavage stages become more or less mingled, although the maturation stages are usually more abundant in the lateral portions of the uterus and the cleavage stages in the middle portions.

[1] Child, C. M., Biol. Bull., XII., 2, 1907.

After passage of the eggs from ovary to uterus has occurred several times the different stages are mingled together in great confusion in the uterus for the uterus is now larger than in earlier stages and the eggs move more freely through it with the contractions of the body. In these proglottids particular stages are not confined to particular regions of the uterus: early maturation and later cleavage stages may occur side by side but even here the earlier stages are more abundant in the lateral and the later in the middle regions of the uterus.

That portion of the oviduct which lies between the entrance of the spermathecal duct and the uterus is highly convoluted and possesses thick walls, but no portion of it functions as a shell-gland. The egg passes into the uterus surrounded only by the vitelline membrane and this often disappears during cleavage.

Moreover, although a well developed vitellarium is present no yolk-cells pass into the uterus with the egg. To all appearances, the vitellarium undergoes degeneration *in situ*, though it is possible that yolk-cells may pass into the uterus in stages later than those which I have observed, or it may be that the reserve supplies of nutriment in the yolk-cells are altered by enzymes and so pass into the uterus in fluid form where they undergo resorption by the embryos.

It is very evident that the transference of the embryos of *Moniezia* to the intermediate host must occur either within the proglottid or in fluid since the absence of a shell or capsule of any kind would exclude the possibility of exposure to the atmosphere.

V. Conclusion.

Maturation and fertilization occur in *Moniezia* in a manner not essentially different from that observed in other forms. Evidently the earlier amitotic history of the germ-cells does not interfere in any way with their properties as germ-cells.

Moreover, although the embryonic development begins with mitosis, at least in most cases, if not in all, this soon gives place in large measure to amitosis, mitosis occurring only occasionally in the larger nuclei in the more slowly dividing regions. Here then, as in the development of the germ-cells, amitosis is appar-

ently connected with more rapid, and mitosis with less rapid division.

The following paper will include a brief account of the rôle of amitosis in the organogeny of the proglottid, together with a general discussion and attempt at interpretation of the data presented regarding amitosis and mitosis in *Moniezia*.

April, 1907.

Explanation of Plate II.

Fig. 1. The entrance of the spermatozoön.

Figs. 2 and 3. Preparation for first maturation spindle; male pronucleus near surface of egg.

Figs. 4, 5 and 6. First maturation spindle.

Fig. 7. Resting stage of oöcyte-nucleus before second maturation division.

Fig. 8. Early stage of second maturation spindle.

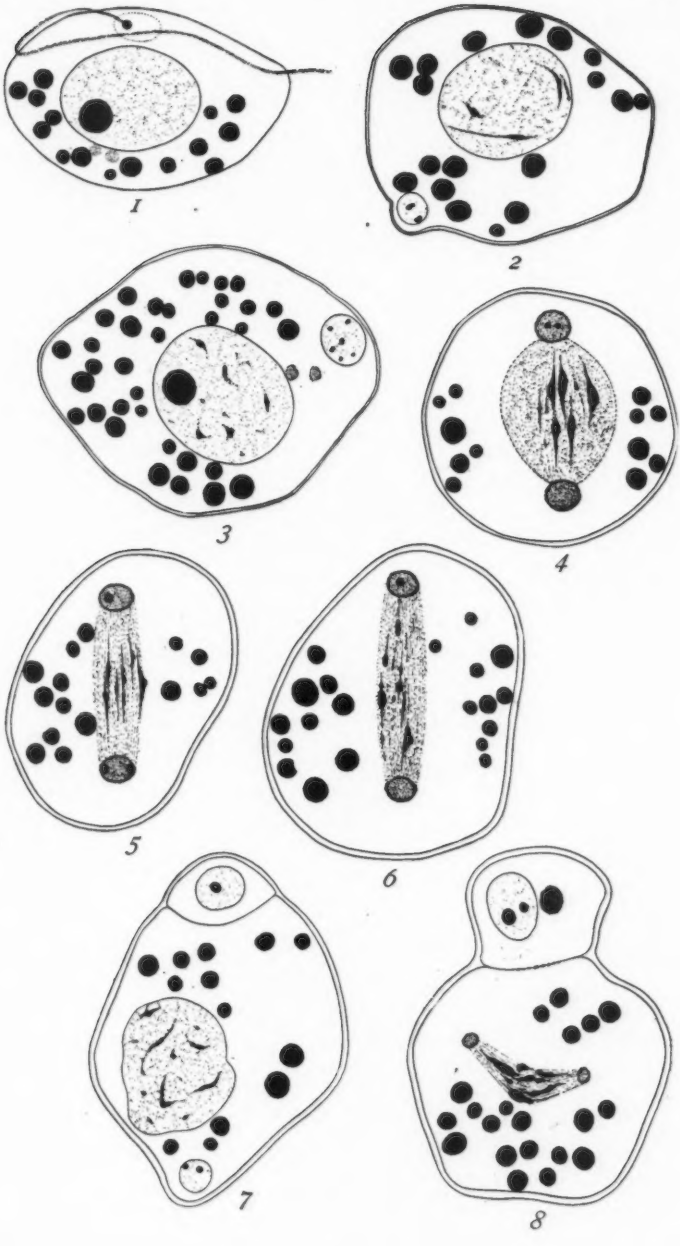
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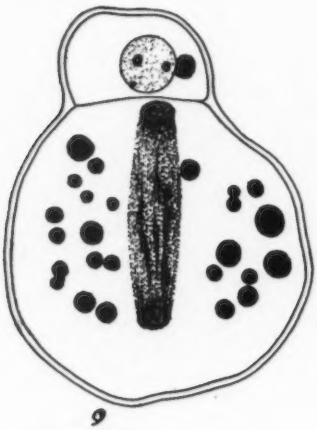
PLATE III.

FIG. 9. Second maturation spindle.
FIG. 10. Second maturation spindle; anaphase, a stage very rarely observed.
FIG. 11. Reconstitution of female pronucleus; male pronucleus on right.
FIGS. 12, 13 and 14. Male and female pronucleus before first cleavage.



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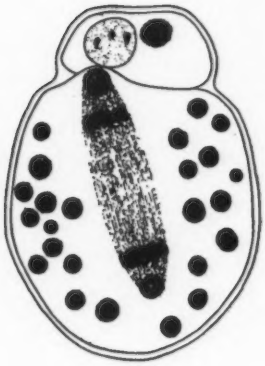

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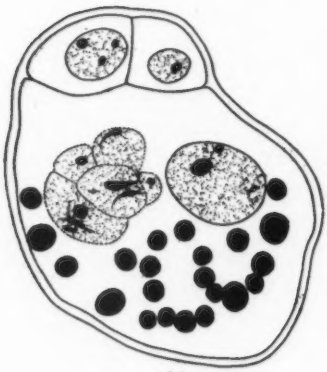

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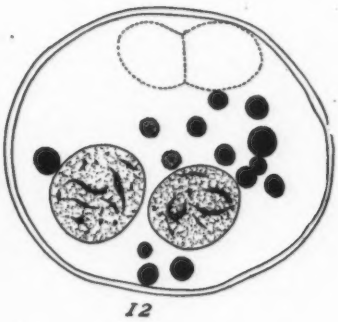

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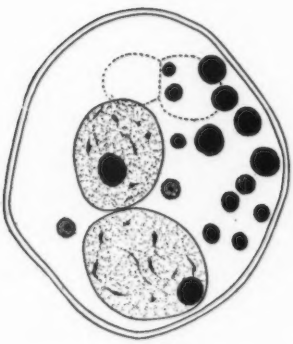

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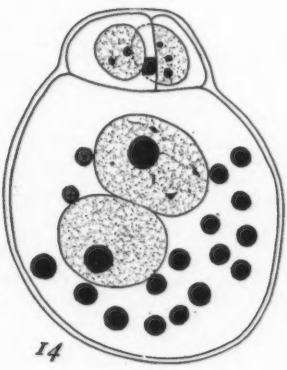

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PLATE IV.

FIGS. 15, 16 and 17. Early stages of first cleavage, showing distinct paternal and maternal groups of chromosomes, in Fig. 17 both within a single nucleus.

FIGS. 18, 19 and 20. First cleavage, with chromosomes in a single group.

The figures show wide variation in the orientation of the first cleavage spindle.

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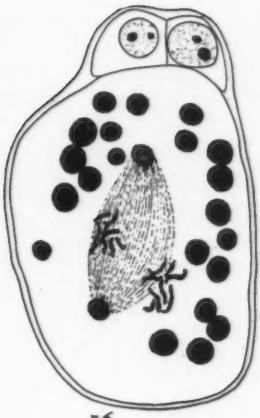

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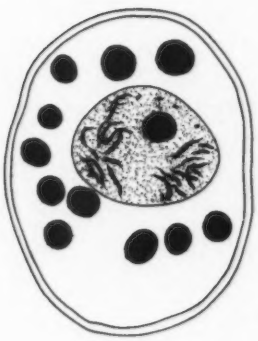

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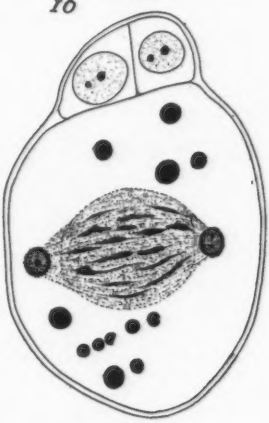

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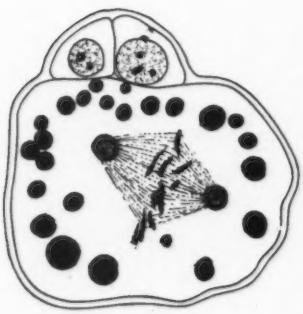

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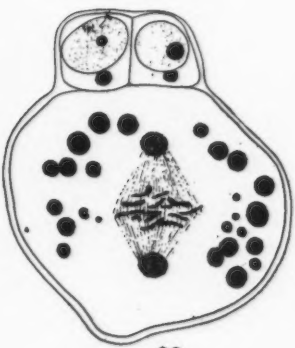

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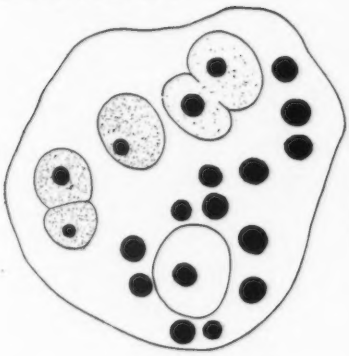
PLATE V.

FIGS. 21–26. Early cleavage-stages.



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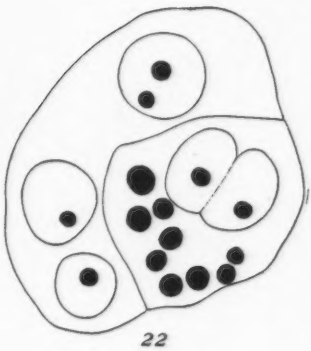

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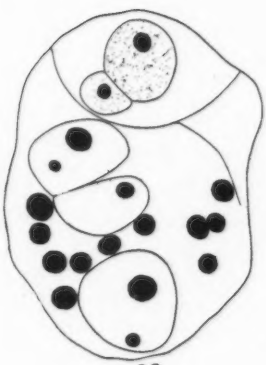

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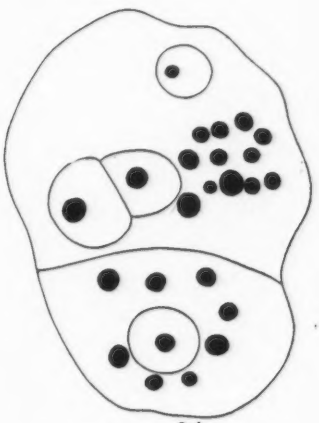

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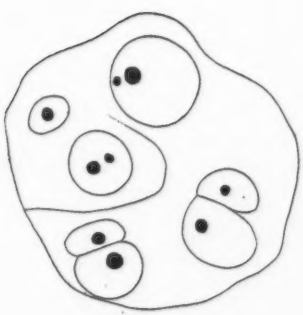

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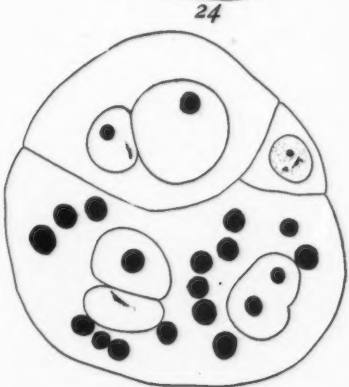

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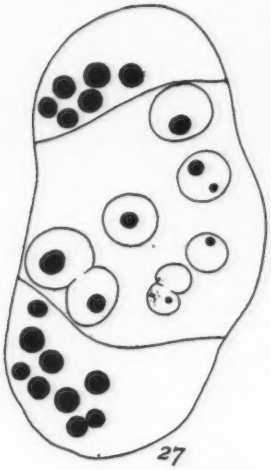
PLATE VI.

FIGS. 27–31. Later cleavage-stages.



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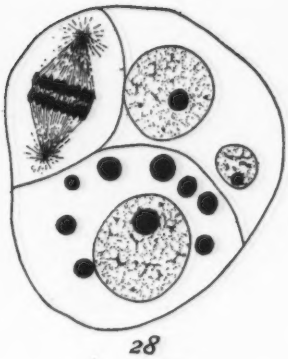

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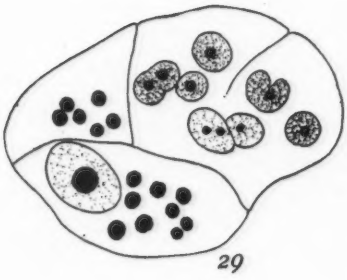

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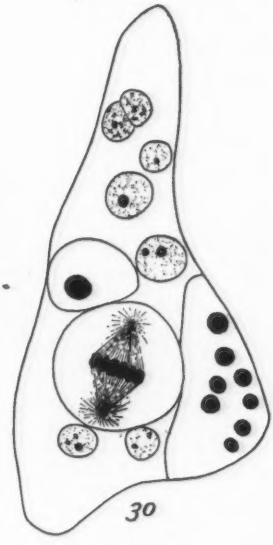

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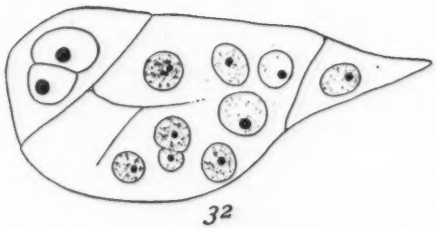
PLATE VII.

FIGS. 32–40. Cleavage-stages; all except Fig. 32 show single blastomeres or dividing nuclei.



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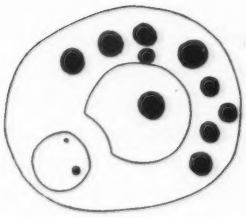

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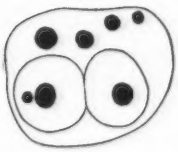

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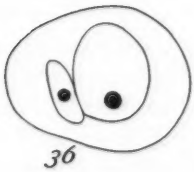

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THE LONGEVITY OF MEMBERS OF THE DIFFERENT CASTES OF TERMOPSIS ANGUSTICOLLIS.

HAROLD HEATH.

Nearly five years have elapsed since I published an account[1] of the breeding habits of three species of California termites, and in this interval it has been possible to add a few facts relating chiefly to the age of the members of the various castes of *Termopsis angusticollis*. As was mentioned in the foregoing account, winged forms do not appear in colonies founded by a primary royal pair until the end of the second year though nymphs, that is larvæ with well developed wing buds, may be recognized bebefore the close of the first year. Also in older colonies immature royal individuals, one or two molts removed from the adult condition, may be found in large numbers in nests from which the winged forms are ready to depart. As there is but one flight a year with this species, it follows that some of the members of the primary royalty are over one year of age before they leave the nest. Furthermore, I have in several instances removed from a flourishing colony a small band of soldiers and well developed workers, together with a number of young individuals which have not undergone more than two molts. The development of these last named insects may be followed without any particular difficulty and where they become true royal forms it is usually after they have been more than one year in the nest.

To determine the length of life of the true royal pair after their mating I partially buried a number of pine logs in a favorable situation and covered them with a cage constructed of fine wire netting. In it were placed, previous to the swarming season, a number of colonies housed in glass jars or naturally founded in logs which had been carried in from the fields. The escape of the winged insects from these nests was normal, and in a short time hundreds of royal pairs were engaged in constructing burrows, which, like the resulting colonies, were developed in the customary fashion as I determined from time to time. During the

[1] "The Habits of California Termites," BIOL. BULL., IV., 47-64.

same period many naturally established nests were located, both at Pacific Grove and about Stanford University, and served to check up results.

In some instances the death of one or both of the royal pair, whether free or in captivity, took place before three years had elapsed; but in certain cases this was undoubtedly due to an unfavorable habitat occasioned by excessive drought or moisture or more often to the ravages of *Termes lucifugus*. Beyond this time their destruction could not so readily be traced to adverse conditions, and may rather be due to exhaustion produced by the arduous duties attendant upon the development of a healthy flourishing progeny. A careful examination showed that at the end of four and one half years the greater number of the kings and queens had died, not over 10 per cent. remaining. After five years I was able to find only six colonies, out of two hundred and thirteen, in which royal forms were present, and but two of these contained both king and queen. In a two-quart fruit jar, which was hermetically sealed and opened only once or twice a year to add water and wood and to remove portions of the continually increasing walls and barricades, I kept a royal pair a few days over five years and eight months. At this time the male died and the queen followed about three months later. From the foregoing it appears that the average life of the royal pair is of at least one year duration in their immature condition, and between four and five after leaving the nest. And further, the life of the male is of practically the same length as that of the female.

To determine the longevity of the workers and soldiers I have in several cases removed from a large colony one or two workers and soldiers which had recently undergone their final molt along with many smaller forms, and have thus been able to distinguish these larger insects from their fellows and to determine their span of life. And again, owing to some slight deformity or some mutilation it has been possible to recognize others in a normally developing colony and to trace their history for years at a time. Also I have taken large communities, headed by true or complemental royal forms, and by removing the young as fast as they appeared, have been able to determine the approximate length of life of all the individuals. From these observations it results

that the workers live about four years after their final molt, and it is probable that this completed state is reached during a period of at least one year, so their life is terminated at the end of about five years.

The soldiers I have been unable to keep, in a great majority of cases, more than three years in a fully developed condition. In a few colonies I have kept them more than four years, and in the nest in the hermetically sealed jar two soldiers lived to be nearly five years of age. Generally speaking the average life of the soldier is about four years from the time of hatching.

While making these observations concerning the formation of the colony and its subsequent development, I have conducted a number of experiments to discover if possible the mode of formation of the various castes. A careful examination of the eggs and the newly hatched young fails to disclose differences which appear to be correlated in any way whatsoever with those distinguishing the soldier, worker and perfect insect to which they give rise. Grassi and Sandias,[1] and others with whom I agree, are of the opinion that it is a question of nutrition ; that the royal pair or the workers by judicious feeding direct the course of development along particular lines. I believe that theoretically all of the young are destined to become perfect insects, but in many cases their growth is arrested or modified and complemental royal forms or soldiers or workers result. Under certain circumstances the modification is not perfect and monstrous forms result, such as soldiers with wings and the ability to produce eggs which may develop. In the examination of many hundreds of colonies formed originally under natural conditions I have found a few such monstrosities, and the surroundings invariably suggest that they have developed under unnatural conditions. In most cases they have appeared in small fragments of wood, which have broken off from the main trunk inhabited by an extensive colony invariably headed by complemental royal forms. With the separation of a small portion of a community changed conditions must arise. New complemental royal forms and in certain cases additional soldiers have to be produced, and it is reasonable to suppose that the enlargement of the nest and the care of the eggs and the developing young demand a profound readjustment

[1] "Costituzione e Sviluppo della Società dei Termitidi," Catania, 1893, 150 pp.

of the conditions obtaining under the old regime. It is accordingly not difficult to imagine that during this transition period the usual mode of feeding of certain individuals may be interfered with and unusual structures result.

In every case these unnatural types are imperfectly developed. For example, the wings of the soldiers are, so far as my experience goes, of less than average size, being in all but two cases less than the length of the abdomen. The reproductive organs likewise are imperfect. In one specimen one half of the ovary was partially functional, the other being in an abortive condition. In two other examples there was an incomplete development of both sides. The mandibles also are of less than average size though within the range of variability which Dr. Desneux writes me is extraordinarily great.

While appearances suggest that these unusual individuals are produced as a result of disturbed conditions, chiefly if not altogether connected with the food and method of feeding, there is no definite proof to show that such is actually the case. For several years I have carried on feeding experiments in the hope that some light might be shed on this important problem, but up to the present time the results are purely negative. I have fed hundreds of immature individuals, which had not undergone more than two molts, entirely or in part upon material carefully removed from the stomach of worker termites and in some instances mixed with fragments of the salivary glands, but such a diet is not perfect, or at all events the mode of feeding is too crude, for the insects soon die and cleared mounts show the alimentary canal to be practically empty. Older individuals live for a longer time on such fare but finally they likewise grow feeble and die. At other times I have fed young and old termites on proctodeal food but the results are never positive. Again I have added to sawdust, upon which these insects thrive, varying quantities of different salts, especially those used in experiments on chemical fertilization, and in other cases have used different acids of various strengths. In other experiments I have mixed the fragmented wood with many substances, nutritious and innutritious intended to disturb the nutritive processes in some degree, but here too, the animal may flourish or starve yet otherwise exhibit no unusual modifications.